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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/501,777	07/19/2004	John Robert Birch	BJS-4145-14	5040

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EXAMINER	
MCGILLEM, LAURA L	
ART UNIT	**PAPER NUMBER**
1636	

DATE MAILED: 11/13/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)
	10/501,777	BIRCH ET AL.
	Examiner	Art Unit
	Laura McGillem	1636

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *26 September 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *1,3-8 and 10-24* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3-8 and 10-24* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *19 July 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☐ Notice of References Cited (PTO-892)

2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)

3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.

4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .

5) ☐ Notice of Informal Patent Application

6) ☐ Other: _____.

DETAILED ACTION

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 9/26/2006 has been entered.

It is noted that claim 1 has been amended. Claims 1, 3-8 and 10-24 are under examination.

Applicants submit that in the Office Action mailed 10/20/2005, claim 2 has been indicated as allowable, and that claim 1 has been revised in the amendment filed 2/21/2006 and 9/26/2006 to clearly include the details of allowable claim 2 (now cancelled) in claim 1 in order to place the application in condition for allowance. Although claim 2 was indicated as allowable in the Office Action mailed 10/20/2005 and claim 1 has been amended to incorporate the limitations of claim 2, on further examination new grounds of rejection were applied, as indicated in the Office Action mailed 5/31/2006.

Claim Rejections - 35 USC § 102

Applicant's arguments, see Remarks, filed 9/26/2006, with respect to claims 1, 3, 7-8, 10, 12 and 19 have been fully considered and are persuasive. The rejection of

claims 1, 3, 7-8, 10, 12 and 19 over Hollis et al (U.S. Patent No. 6,750,041) in view of Abbas et al has been withdrawn.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

Claims 1, 3-8, 10-13, 16, 19-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 99/05267 (Brandt et al) in view of Pu et al (Mol. Biotechnol.1998. Vol. 10, pp.17-25).

Applicants claim a glutamine-auxotropic human cell transfected with (a) an exogenous DNA sequence encoding a protein or an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein, and (b) an exogenous DNA sequence encoding a glutamine synthetase, wherein the sequences are located on more than one DNA construct and capable of producing said protein and growing in glutamine-free medium. Applicants claim glutamine-auxotropic human cells wherein the cells are human fibrosarcoma cells and an HT1080 cell line.

Brandt et al teach an HT1080 cell line which is used to express a target DNA to produce proteins such as EPO, by transfecting a host cell line with DNA constructs including a heterologous expression control sequence which is active in the host cell, wherein the host cell has an endogenous copy of the target gene (see page 5, (a)-(f),

page 6, 2nd paragraph and page 11, Example 2, for example), which reads on a glutamine-auxotropic human cell line or an immortalized fibrosarcoma HT1080 cell line (claims 3-5), which is transfected with an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein. Brandt et al teach that the cells are cultured in serum-free medium and in suspension (see page 8, paragraphs 1.2 and 1.3, for example), which reads on a glutamine-auxotropic human cell where the transfected cell is anchorage –independent and capable of growing in suspension in serum-free medium (as in claim 6). Brandt et al teach that the cells were cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see page 8, paragraph 1.4, for example), which reads on a process for the production of a protein in a glutamine-auxotropic human cell and recovery of the protein (as in claim 7). Brandt et al also teach that the target protein EPO is glycosylated (see page 10, paragraph 1.7, for example), which reads on a cell and a process for production of a protein wherein the EPO protein is glycosylated (claim 8). Claims 10-11 are drawn to serum free and/or glutamine free culture medium used in the claimed process for production of a protein. Brandt et al teaches production of EPO in HT1080 cells grown in suspension cultures in serum free medium. Claims 12 -13 and 16 add limitations to the claimed glutamine auxotropic human cell and process of producing erythropoietin wherein the produced protein is a glycosylated protein and specifically a sialylated protein. Brandt et al teach that it is desirable to have a human EPO protein produced with a comparable glycosylation pattern, especially in regard to sialic acid residues (claims 21-24). Brandt et al do not teach that the glutamine-auxotropic human

cell is transfected with an exogenous DNA sequence encoding a glutamine synthetase for positive selection of glutamine-auxotropic human cells.

Pu et al teach protein expression using glutamine synthetase as a selection marker on a dicistronic expression vector comprising the glutamine synthetase gene and a gene for interleukin-4 receptor transfected into CHO cells grown in glutamine-free medium (see page 18, left column, 1st full paragraph, and right column, paragraph 2.2, for example). Pu et al teach that cells comprising glutamine synthase as a selection marker are grown in glutamine-free medium. Pu et al teach multiple vectors comprising both the glutamine synthetase gene and a gene for interleukin-4 receptor, including a traditional two cassette vector design (pTK-GS-4R), a dicistronic vector with an IRES element between the gene for glutamine synthetase gene and the gene for interleukin-4 receptor (pIRES-GS-4R) and a dicistronic vector with an intron sequence (pINT-GS-4R), see page 20, left column, Fig.1). Pu et al teach that the best protein expression results were achieved using the IRES dicistronic expression vector (see page 22, right column, 3rd paragraph).

The instant specification does not provide a specific definition for the claimed limitation of "DNA construct", so that the skilled artisan would know what elements must comprise the DNA construct in order to be considered a DNA construct. Paragraph 0017 of the Application Publication discloses that the exogenous sequences "are usually located on a DNA construct like an expression vector or an infectious vector". However, this is not a limiting definition of the phrase "DNA construct". Using a broad interpretation of the phase "DNA construct", an expression cassette comprising a gene

or an open reading frame with sequences needed for expression such as transcriptional activating sequences, enhancers and transcriptional termination signals could be considered a "DNA construct". Pu et al teach a glutamine synthetase gene separated from the interleukin-4 receptor gene by the IRES element. Therefore, the portion of the dicistronic expression vector that comprises the glutamine synthetase gene can be considered a DNA construct and the portion of the dicistronic expression vector that comprises the interleukin-4 receptor gene can be considered a second DNA construct, such that the two genes DNA sequences would be located on more than one DNA construct.

It would have been obvious to one of ordinary skill in the art to modify the teachings of Brandt et al to use a dicistronic glutamine synthetase selection system to produce a target protein such as EPO in HT1080 cells because Brandt et al teach that the positive selection marker can be any selection marker suitable for use in eukaryotic cells to convey a selectable phenotype including auxotrophy (see page 5, 4th paragraph, in particular). Pu et al teach that it is advantageous to use an IRES dicistronic expression vector and glutamine synthetase selection over other commonly used selection markers because clones can be selected more rapidly and a wider host cell range can be used. The motivation to use glutamine synthetase selection in HT1080 cells is the expected benefit as suggested by Brandt et al and exemplified by Pu et al of the speed of clone selection and amplification, and a potential wider range of host cells for target protein expression. There is reasonable expectation of success in using glutamine synthetase selection for protein expression in HT1080 cells, since it has

worked before in the cited techniques. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Claims 1, 3-8, 10-13, 16, 19-24 are rejected under 35 U.S.C. 103(a) as being unpatentable over U.S. Patent No. 6,395,484 (Brandt et al, 5/28/2002) in view of Pu et al (Mol. Biotechnol.1998. Vol. 10, pp.17-25). The teaching of Brandt et al and Pu et al are discussed in the above rejection.

Claims 1, 7-8 and 13-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over WO 99/05267 (Brandt et al) in view of Pu et al (Mol. Biotechnol.1998. Vol. 10, pp.17-25) and further in view of Hermentin et al (U.S. Patent No. 6,096,555).

Applicants claim a cell and a process for the production of a protein comprising culturing a glutamine-auxotropic human cell in glutamine free medium and recovering the expressed sialylated protein comprising tri-, tetra- or pentasialo glycoforms. The sialylation is defined by an N-glycan charge.

Brandt et al teach an HT1080 cell line which is used to express a target DNA to produce proteins such as EPO, by transfecting a host cell line with DNA constructs including a heterologous expression control sequence which is active in the host cell, wherein the host cell has an endogenous copy of the target gene (see page 5, (a)-(f), page 6, 2nd paragraph and page 11, Example 2, for example), which reads on a

glutamine-auxotropic human cell line that is transfected with an exogenous DNA sequence capable of altering the expression of an endogenous gene encoding a protein. Brandt et al teach that the cells were cultured to produce the target protein, which was then recovered and quantified from the cell supernatant (see page 8, paragraph 1.4, for example), which reads on a process for the production of a protein in a glutamine-auxotropic human cell and recovery of the protein (as in claim 7). Brandt et al also teach that the target protein EPO is glycosylated (see page 10, paragraph 1.7, for example), which reads on a cell and a process for production of a protein wherein the EPO protein is glycosylated (claim 8). Claims 13 and 16 add limitations to the claimed glutamine auxotropic human cell and process of producing erythropoietin wherein the produced protein is a sialylated glycosylated protein. Brandt et al teach that it is desirable to have a human EPO protein produced with a comparable glycosylation pattern, especially in regard to sialic acid residues.

Brandt et al do not teach that the glutamine-auxotropic human cell is transfected with an exogenous DNA sequence encoding a glutamine synthetase for positive selection of glutamine-auxotropic human cells. Brandt et al do not teach that sialylation is defined by N-glycan charge or that the sialylated protein comprises tri-, tetra- or pentasialo glycoforms of N-glycan.

Pu et al teach protein expression using glutamine synthetase as a selection marker on a dicistronic expression vector comprising the glutamine synthetase gene and a gene for interleukin-4 receptor transfected into CHO cells grown in glutamine-free medium (see page 18, left column, 1st full paragraph, and right column, paragraph

2.2, for example). Pu et al teach that cells comprising glutamine synthase as a selection marker are grown in glutamine-free medium. Pu et al teach multiple vectors comprising both the glutamine synthetase gene and a gene for interleukin-4 receptor, including a traditional two cassette vector design (pTK-GS-4R), a dicistronic vector with an IRES element between the gene for glutamine synthetase gene and the gene for interleukin-4 receptor (pIRES-GS-4R) and a dicistronic vector with an intron sequence (pINT-GS-4R), see page 20, left column, Fig.1). Pu et al teach that the best protein expression results were achieved using the IRES dicistronic expression vector (see page 22, right column, 3rd paragraph).

As discussed in the above rejection, the instant specification does not provide a specific definition for the claimed limitation of "DNA construct", so that the skilled artisan would know what elements must comprise the DNA construct in order to be considered a DNA construct. Paragraph 0017 of the Application Publication discloses that the exogenous sequences "are usually located on a DNA construct like an expression vector or an infectious vector". However, this is not a limiting definition of the phrase "DNA construct". Using a broad interpretation of the phase "DNA construct", an expression cassette comprising a gene or an open reading frame with sequences needed for expression such as transcriptional activating sequences, enhancers and transcriptional termination signals could be considered a "DNA construct". Pu et al teach a glutamine synthetase gene separated from the interleukin-4 receptor gene by the IRES element. Therefore, the portion of the dicistronic expression vector that comprises the glutamine synthetase gene can be considered a DNA construct and the portion of

the dicistronic expression vector that comprises the interleukin-4 receptor gene can be considered a second DNA construct, such that the two genes DNA sequences would be located on more than one DNA construct

Hermentin et al teach a process for characterizing the glycosylation of glycoproteins based on a hypothetical charge number N. Hermentin et al teach that it is important to reliably determine the degree of glycosylation or sialylation in glycoproteins, such as erythropoietin, in order to gauge bioavailability/biological activity of a protein for therapeutic use. Hermentin et al discloses that when erythropoietin is incompletely glycosylated, it is quickly cleared from the blood circulation and would not be biologically useful (see column 1, lines 6-15, 28-45 and column 2, lines 14-25, for example). Hermentin et al teach that it is crucial to determine the distribution of glycan groups exhibiting differing degrees of sialylation to be able to index the bioavailability of a glycoprotein. Hermentin et al teach that the N charge of a glycoprotein is determined in part by determining the percentage of trisialo, tetrasialo and pentasialo ranges (see column 3, lines 27-50, column 4, lines 27-35, column 5, lines 4-12, for example) which reads on a cell-based protein production process wherein the protein is a sialylated protein comprising tri-, tetra- or pentasialo glycoforms of an N-glycan charge (claims 13-18). Hermentin et al teach that the N-glycan charge value was determined for erythropoietin and erythropoietin is comprised of trisialylated N-glycans and tetrasialylated glycans (see column 12, lines 43-52, for example), which reads on erythropoietin as a sialylated protein comprising tri and tetrasialylated glycoforms defined by N-glycan charge and a process of defining sialylation by N-glycan charge.

It would have been obvious to one of ordinary skill in the art to modify the teachings of Brandt et al to use a dicistronic glutamine synthetase selection system to produce a target protein such as EPO in HT1080 cells because Brandt et al teach that the positive selection marker can be any selection marker suitable for use in eukaryotic cells to convey a selectable phenotype including auxotrophy (see page 5, 4th paragraph, in particular). Pu et al teach that it is advantageous to use an IRES dicistronic expression vector and glutamine synthetase selection over other commonly used selection markers because clones can be selected more rapidly and a wider host cell range can be used. The motivation to use glutamine synthetase selection in HT1080 cells is the expected benefit as suggested by Brandt et al and exemplified by Pu et al of the speed of clone selection and amplification, and a potential wider range of host cells for target protein expression. There is reasonable expectation of success in using glutamine synthetase selection for protein expression in HT1080 cells, since it has worked before in the cited techniques.

It would have been obvious to the skilled artisan to determine the N-glycan charge for the erythropoietin because Hermentin et al teach that it is important to know the degree of glycosylation of recombinant therapeutic proteins such as erythropoietin, since slightly altered glycosylation patterns can drastically effect the activity of the therapeutic protein. The motivation to do so is the expected benefit of being able to determine the degree of glycosylation in a simple reliable manner suitable for replacing the methods previously known in the art for determining the bioavailability of a therapeutic protein before use. There is reasonable expectation of success in combining

the methods of Brandt et al, Pu et al and Hermentin et al to use a glutamine- auxotropic human cell transfected with an exogenous DNA sequence encoding a glycoprotein, such as erythropoietin, to produce and recover erythropoietin and determine its bioavailability via N-glycan charge, because these methods have worked before in the cited references. Given the teachings of the prior art and the level of skill of the ordinary skilled artisan at the time the invention was made, it must be considered that said ordinary skilled artisan would have had a reasonable expectation of success in practicing the claimed invention.

Conclusion

No claims are allowed.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Laura McGillem whose telephone number is (571) 272-8783. The examiner can normally be reached on M-F 8:00-5:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Irem Yucel can be reached on (571) 272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only.

For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Laura McGillem, PhD
10/27/2006

DANIEL M. SULLIVAN
PATENT EXAMINER